MOTILITY OF *HUCHO HUCHO* L. SPERMATOZOA AFTER ACTIVATION

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Observations of the Danube salmon spermatozoa motility and spermatocrit were conducted. Sperm was tested immediately after spawning and during its prolonged storage.

**INTRODUCTION**

The specialized literature, so far, has no data on complex studies of *Hucho hucho* L. spermatozoa and conducted surveys were fragmentary only. Haempel (1913), for example, had observed motility of *Hucho hucho* L. spermatozoa since their activation in water and noted it to last up to 45 sec. According to Scheuring (1928), time when *Hucho hucho* L. spermatozoa stayed motile reached up to 76 sec (progressive motion up to 30 sec.) depending on the spermatozoa quality; getting shorter with prolonged storage of sperm. Pavlik (1957) confirmed Haempel’s results, while Harsanyi, cited by Holčík et al. (1984) found the *Hucho hucho* L. spermatozoa to be motile up to 120 sec after their activation in water of 10°C. Analysis of Danube salmon spermatozoa with electron microscope proved their structure to be typical for salmonids, with lack of acrosome and eccentric situation of the flagella in relation to the head (Radziun and Tomasik, 1985).

Difference in conclusions, in just few papers dealing with the *Hucho hucho* L. spermatozoa motility problem, induced us to undertake complex, to some extent,
studies. Besides we wanted to find out if and to what extent observations done by others on fish spermatozoa motility (Nomura, 1964; Yoshida and Nomura, 1972; Holtz et al., 1977), dividing whole motility period into separate phases of progressive motion and pendular motion, can be applied to *Hucho hucho* L. spermatozoa.

**MATERIAL AND METHODS**

Surveys were conducted in 1985 in the Polish Anglers Association Station at Łopuszna and then repeated in 1988. Sperm from 11 and 6, respectively, mature *Hucho hucho* L. males was analysed. Males, whose ejaculates were tested, were measured and their age determined.

Surveys included determination of total motility time of spermatozoa after getting in touch with water, distinguishing the length of each phase of the motion; namely — progressive motion random (turbulent) and circular (quiet) — according to motion’s intensity and pendular motion (Fig. 1). For all samples a relative number of active spermatozoa (percent of motility) and a relative volume of spermatozoa within a sperm sample = ”spermatocrit” according to the method by Winnicki and Tomasik (1976), were determined.

![Fig. 1. Motion of *Hucho hucho* L. spermatozoa after activation (explanation in text)](image_url)

A total time of spermatozoa motility was number of seconds from an activation moment up to time when there was not more than 5% spermatozoa with pendular motion within the sample. A progressive motion time started with activation process and lasted to the moment when number of spermatozoa with this type of motion dropped below 50%. Limit of a random (turbulent) phase was determined analogically. Difference between total motility time and progressive motion time was pendular motion time (Fig. 1). Number of active spermatozoa was determined on spermatozoa transition from random (turbulent) phase into a circular (quiet) one.

The spermatozoa motility tests, according to the method by Tomasik (1973) were conducted with optic microscope (enlargement 200 x). Time of each phase was measured with stoper with 0.5 sec accuracy. Observations were carried out in a cold room, at the temperature around 5°C, the same as the one in water tanks, were *Hucho hucho* L. males were being kept (to avoid heating up a sample while on microscope stage).

Spermatocrit was estimated by hematocrit centrifuge (5000 rpm; for 5 min.). Each result of all the tested parameters for each individual is an average of three successive measures.
Some of the ejaculates were transported, in cooled thermos container (temp. while transported 5°C±1°C), to the laboratory at the Institute of Ichthyology, where surveys on spermatozoa motility in the tap water, at +8°C were being continued. Obtained results were worked out statistically.

RESULTS

Spermatozoa tested were distinguished by relatively high activity (about 70% of active spermatozoa) with spermatocrit average being 16.7% (Tab. 1). Progressive motion of spermatozoa, extended in time, lasted about 48 sec., where 26 sec was an average time for preliminary phase of that motion — a random (turbulent) one (that is 55% of a total translatory motion). Progressive motion was then replaced by a relatively long lasting pendular motion (84%, that is 64% of total motility time of spermatozoa).

Table 1

<table>
<thead>
<tr>
<th>Specification</th>
<th>Longitudo caudalis (cm)</th>
<th>Age (years)</th>
<th>Spermatozoa activation (%)</th>
<th>Motion time (sec.)*</th>
<th>Spermatocrit (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>10</td>
<td>17</td>
</tr>
<tr>
<td>X</td>
<td>81.7</td>
<td>7.9</td>
<td>69.0</td>
<td>26.1</td>
<td>47.9</td>
</tr>
<tr>
<td>SE</td>
<td>3.6</td>
<td>0.6</td>
<td>5.4</td>
<td>2.2</td>
<td>3.4</td>
</tr>
</tbody>
</table>

* spermatoza motion time measured since activation moment

Interesting data were provided by surveys on the spermatozoa motion phases after 24 and 48 hours cold storage (+5°C) of sperm samples, transported then to the laboratory in Szczecin (Tab. 2). Since 24 hours of cold storage a gradual reduction of progressive motion and pendular motion periods was observed, down to about half of its prime value after 96 hours of storage. After 216 hours spermatozoa of only one male showed signs of activity. There was not any signs of a random (turbulent) phase of spermatozoa motion observed already 24 hours after the ejaculates collection. Quite possibly this phase, then, is too short to observe it (within few seconds necessary to get ready for microscopic observations).
Motility of Danube salmon, *Hucho hucho* L. spermatozoa, when stored

<table>
<thead>
<tr>
<th>Time since spermatozoa collection (h)</th>
<th>Specification</th>
<th>Spermatozoa activation</th>
<th>Motion time (sec.)* ***</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td></td>
<td>progressive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>random (turbulent) phase</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>circular (quiet) phase</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>pendular</td>
</tr>
<tr>
<td>24*</td>
<td>n</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>X</td>
<td>28.2</td>
<td>25.1</td>
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<tr>
<td></td>
<td>SE</td>
<td>5.96</td>
<td>4.3</td>
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<tr>
<td>48*</td>
<td>n</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>X</td>
<td>21.5</td>
<td>32.3</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>5.18</td>
<td>2.54</td>
</tr>
<tr>
<td>96**</td>
<td>n</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>X</td>
<td>34.3</td>
<td>24.5</td>
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<tr>
<td></td>
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<td>1.58</td>
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<tr>
<td>144**</td>
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<td>6</td>
<td>6</td>
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<tr>
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<td>X</td>
<td>24.8</td>
<td>24.8</td>
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<tr>
<td></td>
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<td>1.74</td>
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<td>2</td>
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<tr>
<td></td>
<td>X</td>
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<td>20.5</td>
</tr>
<tr>
<td>216**</td>
<td>n</td>
<td>1</td>
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</tr>
</tbody>
</table>

* surveys at the spawning ground  
** surveys after transportation to the laboratory  
*** spermatozoa motion time measured since activation moment

**DISCUSSION**

Observations done, so far, on time of activated *Hucho hucho* L. spermatozoa motion were not numerous and not too precise. Authors were interested only in the spermatozoa motion itself, not mentioning its character and phases. Scheuring (1928) was the only one, who had taken into consideration a progressive motion and pendular motion of *Hucho hucho* L. spermatozoa.

Our observations, repeated twice, in long intervals, on individuals from the same environment, under the same conditions, indicated the motility time of *Hucho hucho* L. spermatozoa to last up to 230 sec. with the average motility time being 132 sec. That is longer than it was observed by Scheuring (1928) and Harmanyi cited by Holák et al.
According to Smirnov (1954, 1963, 1975) progressive motion of pink Salmon (Oncorhynchus gorbyscha) spermatozoa oscillated between 30 and 55 sec., while Caga and Chorevina (1984) found it to last up to 80 sec., at temp. 6 to 9°C. Spermatozoa of Oncorhynchus keta were in progressive motion for 10 to 15 sec. after their activation in water of temp. 10°C (Smirnov, 1975). The same type of motion for spermatozoa of Salmo gairdneri lasted 23 sec. (Billard, 1978) while for Salmo trutta average progressive motion time was 42 sec. (Szymelfenig, 1979).

Our experiments let us to distinguish two phases within a progressive motion; a random (turbulent) phase with rapid straight forward movements of spermatozoa and a circular (quiet) one where the motion visibly slows down and enables to distinguish individual spermatozoa in rectilinear motion and then rotary one. That rotary motion can result from eccentric situation of flagella to the head of spermatozoa (Turdakov, 1972; Radian and Tomaszik, 1985).

Lately conducted surveys on Thymallus thymallus let to distinguish also, two phases within a progressive motion (work in print). However, a random (turbulent) phase is very short (few seconds), while the same phase for Hucho huchu salmon took longer (20 to 30 sec.) and was visibly pronounced. We are to admit, that differences in time of spermatozoa motion measured by Scharpf (1912) and Povil (1957) on one side and Harcanyi (Hobik et al., 1964) and us on the other were, probably, due to the progressive motion measures taken only by the first. According to Schering (1928) a progressive motion time was 30 sec., with total motion time (76 sec.) somewhat shorter than the one we noted. However he transported his sampler in container filled with ice, while our experiments were conducted immediately after spawning, in the same water fish males were taken from, that is almost in natural conditions. Only the second part of the experiment (Tab. 2) was conducted on sperm transported to Ekaterin.

Our surveys proved the total progressive motion time for Hucho huchu spermatozoa is somewhat longer than for other salmonids, being similar to the one for pink salmon. The only exception found was Salmo gairdneri for whom that type of motion are relatively very short. A particularly long pendular motion time was characteristic for Hucho huchu spermatozoa.

Production volume of Hucho huchu spermatozoa in sperm (spermatocrit) is rather low when compared with other salmonids. Wunderlich and Tomaszik (1976) stated, for example, the spermatocrit of mature Salmon stroma males to range from 20 to 50%. According to various authors spermatocrit value of Salmo gairdneri ranged from 10 to 40% (Kanitz and Jacobson, 1972; Tomaszik et al., 1981; Pihola and Hyvärinen, 1983).

These essential differences and sometimes surprisingly high spermatocrit values (up to 40 and more %) can be presumably referred to differences in method used to collect sperm for surveys. It is known, that “testing” males have “more diluted” sperm than males artificially engorged when mechanical pressure is applied.
Hence, for the mature Danube salmon (*Hucho hucho*, L.) males percent of spermatozoa activation is rather high (70%), while spermatocrit relatively low (17%). Immediately after spawning a random (turbulent) phase of progressive motion is clearly distinguished and the total motion time of spermatozoa equals to 130 sec. During cold storage of sperm, spermatozoa stay viable for a long time.

REFERENCES


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Motility of Hucho spermatozoa after activation


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STRESZCZENIE

W latach 1985 i 1988 przeprowadzono obserwacje ruchliwości i spermatokrytu plemników 17 samców głowacicy (Hucho hucho L.). Stwierdzono, że względna ilość aktywnych plemników w spermie jest znaczna (średnio 70%), a spermatokryt (względna objętość plemników w spermie) niski (średnio 16.7%) w porównaniu z innymi rybami łososiwatymi. Bezpośrednio po tarle, wśród zaaktywowanych plemników wyraźnie zaznaczona jest faza burzliwa ruchu postępowego. Całkowity czas ruchu plemników wynosi średnio 130 sek, z czego na ruch postępowy przypada ok. 37% (śr. 48 sek.).

Plemniki w spermie przetrzymywanej w temp. 8°C zachowują żywotność do 216 godz.

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